STUDIES RELATIVE TO THE APPARENT CLOSE RELATIONSHIP BETWEEN BACT. PERTUSSIS AND B. BRONCHISEPTICUS[1]

II. COMPLEMENT FIXATION TESTS

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In a previous article (Ferry and Noble, 1918) we have described the cultural, agglutination and absorption reactions between *Bact. pertussis* and *B. bronchisepticus* and have shown that, while the two organisms are distinct, they are apparently somewhat closely related. The most striking characteristics of the organisms, according to the serological reactions, were shown to be the ability of *B. bronchisepticus* to produce an immune serum that would agglutinate both the *B. bronchisepticus* and *Bact. pertussis* antigens and the ability of *Bact. pertussis* to produce an immune serum that would agglutinate only the homologous antigen. The absorption reaction showed that the *B. bronchisepticus* antigen would absorb from the anti-bronchisepticus serum (a serum that contained agglutinins for both organisms) only the *B. bronchisepticus* agglutinin (the major agglutinin) leaving intact the agglutinin for *Bact. pertussis* (the minor agglutinin). This minor agglutinin could only be absorbed by the *Bact. pertussis* antigen. This type of an agglutinin was termed by the authors a "transitive" agglutinin.

The present investigation was undertaken to confirm the work of the previous paper through complement fixation tests and to determine, if possible, the value of this test in differentiating between the two organisms.

Strains used. At first a large number of strains of each organism were used, the same strains as those worked with in the pre-

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vious experiments already described, but as it was found that all strains of the same organism gave similar reactions it was deemed advisable to cut down the number to three of each in order to save time. Of the *B. bronchisepticus*, no. 36 (dog), no. 123 (monkey) and human strains were tested; and of *Bact. pertussis*, no. 0363 (Bordet), no. 109 and no. 248 (Povitzky).

Technic. For the volume of the complement fixation tests it was found more satisfactory to use 2 cc. than 5 cc. as advised for the Wassermann test or 0.5 cc. suggested by Olmstead and Povitzky in a serological comparison of the Bordet-Gengou bacillus and hemoglobinophilic bacilli. The hemolytic system was composed of sheep cells in a 2 per cent suspension, guinea-pig complement in a 1 to 10 dilution and rabbit amboceptor in 1 to 1500 dilution. Complement titration was made by using 0.1 cc. of amboceptor and varying amounts of complement.

In determining the relationship of the various strains two units of both amboceptor and complement were employed. All titrations were incubated one hour before and one hour after the addition of the sensitized cells, at 32°C. The dilution was chosen in which complete hemolysis was produced, readings being made at the end of the hour's incubation. All serum was inactivated by heating in water bath one half hour at 56°C.

The antigen was titrated by mixing it in varying amounts with one unit of the hemolytic system.

Preparation of antigen. After trying out several methods of antigen preparation it was finally determined that filtered autolysates gave the most stable and satisfactory products.

The antigens were prepared as follows: The organisms were grown on agar for forty-eight hours at 37.5°C., then taken off and suspended in distilled water and shaken for forty-eight hours in a mechanical shaker. This suspension was then heated at 56°C. for one-half hour, incubated twelve hours, after which enough sodium chloride and formalin was added to make an 0.85 per cent and 0.5 per cent solution respectively. Filtration was carried on through asbestos.

Preparation of immune serum. The same serums were used for this work as for the previous experiments, a description of which has already been given.

The results of the complement fixation tests may be seen in the following table:

ANTIGENS	ANTISERUMS					
	B. bronchisepticus (dog) no. 36	B. bronchisepticus (monkey) no. 123	B. bronchisepticus (human)	Bact. pertussis no. 0363	Bact. pertussis no. 109	Bact. pertussis no. 248
B. bronchisepticus (dog) no. 123	+	+	+	−	−	−
B. bronchisepticus (monkey) no. 123	+	+	+	+	+	+
B. bronchisepticus (human)	+	+	+	+	+	+
Bact. pertussis no. 0363	+	+	+	+	+	+
Bact. pertussis no. 109	+	+	+	+	+	+
Bact. pertussis no. 248	+	+	+	+	+	+

+ Denotes complete inhibition of hemolysis.
− Denotes incomplete or no inhibition of hemolysis.

It was found in a large majority of the tests as represented in the chart, which is a composite, that the *B. bronchisepticus* immune serum bound the complement in the presence of both the bronchisepticus and pertussis antigens, while the *B. pertussis* immune serum bound the complement in the presence of the homologous antigen and also the human and monkey strains of *B. bronchisepticus*. It did not bind the complement in the presence of a dog strain of *B. bronchisepticus*.

SUMMARY

1. *B. bronchisepticus* immune serum bound the complement in the presence of both *B. bronchisepticus* and *Bact. pertussis* antigen.

2. *Bact. pertussis* immune serum bound the complement in the presence of *Bact. pertussis* antigen and *B. bronchisepticus* antigen of both human and monkey origin but not of dog origin.

3. The complement fixation test is not a reliable method of differentiating between the two organisms in question.

4. Bacterial autolysates were found to be the most stable and satisfactory antigens.

5. The complement fixation test was found to corroborate, in most respects, the agglutinin reactions reported in a previous paper.

REFERENCES

FERRY, N. S. AND NOBLE, ARLYLE 1918 Studies relative to the apparent close relationship between Bact. pertussis and B. bronchisepticus. I. Cultural, agglutination and absorption reactions. J. Bact., Balt., **3**, 193.